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PRINCIPAL INVESTIGATOR: Konstanty Mazuruk, Ph.D.

CONTRACTING ORGANIZATION: University of Alabama at Huntsville
Huntsville, Alabama 35899-0001

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Konstanty Mazuruk, Ph.D.

E-Mail: consty.mazuruk@msfc.nasa.gov

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14. ABSTRACT

The research goal of this project was to develop a novel class of magnetic fluids that exhibit thermo-regulating properties attractive for a number of medical applications. These fluids could potentially surpass the functionality of currently available magnetic fluids used in hyperthermia A A novel class of nano-particles has been discovered that possesses an inherent self-limiting mechanism against overheating and it utilizes a different physical principle than the Curie point based principle. These systems have almost ideal for magnetic fluid hyperthermia self-limiting heating properties.

15. SUBJECT TERMS
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INTRODUCTION

Magnetic particles with micron or sub-micron dimensions are now becoming highly attractive for many biomedical applications, such as targeted drug delivery, gene therapy, disease detection, biochemical sensing, genetic screening. Advances in these areas are largely due to the research progress in nano-technology and in magnetic fluid technology, in particular. The last one gave birth to the magnetic fluid hyperthermia (MFH) - an important tool for cancer treatment.

Hyperthermia is a cancer therapy consisting of heating a tumor region to the elevated temperatures in the range of 42-45 ^oC for an extended period of time (2-8 hours). This leads to thermal inactivation of cell regulatory and growth processes. Moreover, heat boosts the tumor response to other treatments such as radiation, chemotherapy or immunotherapy. Of particular importance is careful control of generated heat in the treated region and keeping it localized. Higher heating, to about 56^o C can lead to a healthy tissue thermo-ablation. With accurate temperature control, hyperthermia has the advantage of having minimal side effects. Several heating techniques are utilized for this purpose, such as whole body hyperthermia, fever induced hyperthermia, radio-frequency (RF) hyperthermia, ultrasound technique, microwave hyperthermia, inductive needles (thermo-seeds), and magnetic fluid hyperthermia (MFH).

Magnetic fluid hyperthermia offers many advantages such as targeting capability by applying magnets. However, this technology still suffers significant inefficiencies due to lack of thermal control. Nano-particles with inherent thermoregulating properties may solve this problem. The concept of thermoregulating particles for hyperthermia is an approach where an alternating magnetic field is used to excite magnetic particles and generate heat. It is novel in that it has a built-in safety switch – a low Curie temperature (temperature at which magnetic properties are lost) that limits the maximum temperature. Although there are known many magnetic systems with the desired Curie temperature (43^oC), a number of additional requirements has to be fulfilled. This includes non-toxicity, fast removal from the organism, high absorption of RF power, good targeting capabilities, good MRI contrast. In order to develop a nano-material, competitive to the present state-of-the-art Fe_2O_3, a significant amount of research is needed in several areas, such as nano-fabrication technology, physics of magnetism, numerical thermal modeling, MRI thermo-tomography, toxicology, medical trials, etc.

The research goal of this project was to develop a novel class of magnetic fluids that exhibit thermo-regulating properties attractive for a number of medical applications. These fluids could potentially surpass the functionality of currently available magnetic fluids used in hyperthermia. The specific goals are:

1. Develop a synthesis process to fabricate magnetic nano-particles with adequate characteristics for thermo-regulating magnetic fluid (TRMF) operating in 40-45^o C range.
2. Test and characterize TRMF using microscopy, diffraction and spectroscopy techniques such as powder XRD diffraction, dynamic light scattering, FTIR and HRTEM, SAR measurements, magnetic susceptibility measurements, etc.
3. Develop a thermal breast model with the distributed TRMF and determine the volumetric thermal field characteristics in the presence of RF heating.
4. Evaluate performance and advantages of the TRMF as compared to the ordinary MF.
5. Evaluate efficacy of the thermo-regulating magnetic fluid in breast cancer hyperthermia.

All the above goals have been achieved in the 2003-2006 year research effort, and the additional requested no-cost extension time was devoted to the critical appraisal of the results, writing final report, and seeking new funding opportunities for continuation of the initiated research.

BODY

The reporting activities during this extended period include

1. Compilation of all acquired research data, and writing a final report.
2. Seeking new funding opportunities for continuation of the proposed research.

During the course of this research, several other groups have attempted to develop magnetic nano-particles with thermo-regulating properties that could potentially be used for hyperthermia. However, data published by others and our own results from this DoD BCRP IDEA project have shown that the transition from the high to low power absorption states is rather broad. Therefore, we believe that this approach may not be suitable for MFH. The rationale, why the magnetic nano-material operating near the Curie point is inadequate for MFH is as follows. For MFH, heating up from 37^{o}C by only 6^{o}C is required. Ideally, magnetization has to be as high as possible at 37^{o}C to generate heating efficiently. Then it is desirable that it drops down to zero above 45^{o}C, so that RF absorption will become very low. However,

(1) magnetization is small near the Curie point, so that the heating efficiency is also small. High RF power is then required to generate a therapeutic temperature level. However, biologically dangerous level of RF power may be reached.

(2) change in the magnetization within the narrow temperature range of 6^{o}C is not considerable, so that the thermo-regulating effect is rather modest.

The research efforts to find a good thermo-regulating nano-material among ferro- or ferri-magnetic systems undergoing the Curie transition have been so far unsuccessful. As a result of our research efforts, we believe, we have found a new technology that adequately addresses the thermal uniformity issue of MFH. A novel class of nano-particles has been discovered that possesses an inherent self-limiting mechanism against overheating and it utilizes a different physical principle than the Curie point based principle. A small class of magnetic systems exists that exhibits the first order magnetic transition (FOMT) from the ferromagnetic to the paramagnetic state. Being a structural transition of first order, it is dramatically sharp and occurs within one to two degrees Celsius. RF power absorption of this material will switch from the high absorption state to the low state within 1-2^{o}C at the FOMT temperature. Magnetic nano-particles formulated from such systems should have almost ideal for MFH self-limiting heating properties. By tailoring the composition of the nano-particles, the FOMT temperature can be brought within the hyperthermia treatment range of 42-45^{0}C. We have recently synthesized nano-particles of one such composition and demonstrated a temperature saturation at 44^{0}C during RF heating. The provisional patent application for this new class of magnetic nano-particles has been filed with the US Patent Office [1]. MFH based on FOMT type nano-particles is envisioned as a new breakthrough technology in hyperthermic treatment of breast cancer.

Fig.15. MnAs ferrofluid in a static magnetic field.

Fig.16. Gold nanocolloid made by the laser ablation method (left-in heavy water, right- in ordinary water).

Consequently, we have narrowed our research to the class of ferromagnetic materials that exhibit the first order transition. Some of the magnetic semiconductors exhibit such an uncommon behavior. For further study, we have selected MnAs. It exhibits the first order magnetic transition (FOMT) at 44^{o}C, which is optimal for the hyperthermia. The chemical synthesis of the nanoparticles of one of pnictides (MnP)has been recently demonstrated [14]. MnP is chemically similar to MnAs compound. However, it is a difficult method, and yields only non-spherical nanoparticles. Nanoparticles of MnAs have not been reported at this point. Instead, we have utilized a traditional method for a ferrofluid preparation, namely, ball milling.

The charge of 20 grams of MnAs particles in 500mL of hexane, with the oleic acid as a surfactant, has been ball-milled for 1 month yielding MnAs nanoparticles of 80-100nm diameter. The diluted nanocolloid fluid in hexane maintains stability for over one month. The concentrated ferrofluid displays a typical spike behavior when a magnet is placed in its proximity (see Fig.15).

The toxicity issue is critical for development of biocompatible nanoparticles. Although only a small amount of nanoparticles (20mg) is required for hyperthermia, highly toxic material is not adequate for medical applications. Nickel and its metal-organic compounds, for example, are toxic. Capping with an inert layer, such as a gold coating, can be considered as a remedy. However, the body system will not be able to chemically decompose the nanoparticle. Thus the nanoparticles may accumulate in some parts of the body. MnAs in this regard has not been studied in biological systems. Generally, manganese is non-toxic. Metallic arsenic and its inorganic compounds can be toxic. It occurs naturally in water. On the other hand, organic arsenic is non-toxic, as a rule. Its organic chemistry is similar to organo-phosphides. Humans consume significant quantity of organo-arsenic with fish (100mg). Arsenic is not accumulated in body, contrary to heavy metals. Considering that MnAs is a very stable chemical compound, it is expected that its toxicity level is small, and it can be slowly and completely eliminated from the body. However, experimental work in this direction has to be performed in order to consider it for hyperthermia or other medical applications. Note that a number of other compounds exhibiting the FOMT transition do exist, some phosphides, for example.

Following advances in synthesis of nanoparticles in the last years, we have investigated the synthesis method based on thermal decomposition of metal-oleate complexes. At this point, this method has been reported only for iron nanoparticles [15]. We have demonstrated its utility to fabricate nickel, tungsten, zinc, and copper nanoparticles. It is amenable to fabricate many alloys as well. The extension of this method to synthesize pnictides would be a breakthrough in this direction.

We have extended the oleate complex decomposition method to utilize the oleylamine as a complexing agent. An advantage would be the presence of nitrogen at the surface, resulting in a protective nitride shell. In fact, nickel nanoparticles, synthesized by this modified technique in our laboratory, have been stable in air for several months.

We have investigated a laser ablation concept to fabricate nanoparticles [see the attached subcontract report]. We have applied for the patent for this method [2]. The technique is particularly suitable for noble metals, as it yields nanocolloids in a few minutes with no chemical impurities present. The fabrication of nanocolloids made of compounds may, however, be problematic, as a chemical decomposition of the material may occur.

MODELING WORK

In order to theoretically demonstrate the advantage of the novel magnetic nanoparticles for hyperthermia, we have developed a thermal model of a breast tumor MH. The model is based on the bioheat equation proposed by Pennes:

$$\rho c \frac{\partial T}{\partial t} = \nabla (k \nabla T) - c_b w (T - T_b) + A + Q$$

where c - is specific heat,
κ –thermal conductivity,
w –blood perfusion rate,
A- metabolic heat rate,
Q-electromagnetically induced heat rate,
T_b-blood temperature,
c_b-specific heat of blood,
ρ - tissue density.

$$\frac{k}{\rho c} = 1.381 \times 10^{-7}\, m^2/s$$

$$\frac{A}{\rho c} = 1.326 \times 10^{-4} Hz$$

$$q = \frac{Q}{\rho c} = \frac{0.2}{e^{(T-40)} + 1} e^{-0.1 r^2}$$

$$\frac{c_b w}{\rho c} = \begin{cases} 9.66 \times 10^{-4} \left(0.45 + 3.55 e^{-(T-45)^2/12}\right) Hz \\ 5.1 \times 10^{-4} Hz \end{cases}$$

$$T_b = 37\,^0C$$

Note that the bio-heat equation is non-linear: blood perfusion and heat generation are temperature dependent.

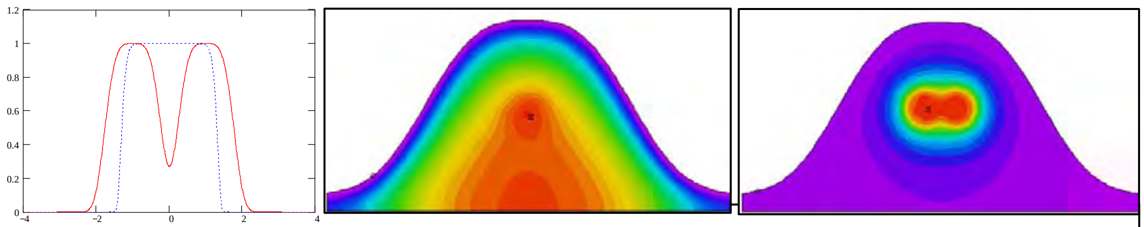

Fig.20. a) particle distribution (red) for modeling, tumor region (dotted blue); b) temperature distribution with no RF heating present; c) RF induced temperature.

When the nanoparticles are uniformly distributed over the tumor region, the temperature reaches 44^{o}C everywhere in this area, provided the RF heating power is sufficient. In case of poorly distributed nanoparticles, the temperature still can vary significantly. Here we consider a tumor localized in the volume o radius 1.5cm as depicted in Fig.20a (dashed line). Now assume that nanocolloid injections were administrated only in two opposite spots at the tumor periphery, so that the particle distribution along the centerline between these spots is represented by the solid line in Fig.20a. With no RF heating present, the thermal distribution is displayed in Fig.20b. The temperature varies insignificantly, by less than 0.3^{o}C, due to the body heat generation. The tumor area is slightly hotter due to the perfusion rate difference between the tumor and healthy tissue. The RF heating of dispersed thermoregulating nano-colloid with the properties of MnAs, as shown in Fig. 20c, will create a significant thermal distribution, but with no overheating beyond 45^{o}C. However, the temperature varies only by 0.5^{o}C along the line passing through the two injection points, within tumor area. The highest variation in temperature across the tumor region is 1^{o}C. In conclusion, even poorly distributed thermoregulating nanoparticles could perform quite well in terms of a thermal profile. Practically, the nanoparticles have to be distributed evenly along the perimeter of the tumor, and also near the arteriole vessels entering the tumor region.

RF HEATING WORK

Fig.21. A hyperthermia breast cancer demonstration setup.

The RF power system has been built and used for this work. The frequency range of the system is 100 - 450 kHz and the power is 3kW. The impedance matching circuitry has been designed and fabricated. The SAR test measurements have been done on several nanocolloid systems. The technique consists of imposing an intermittent sequence of the RF heating cycles and measuring the temperature response. The SAR can be calculated from the slopes at each beginning of the cycle [see Fig.22].

The SAR curve obtained for the MnAs nanocolloid is displayed in Fig.23. A sharp drop in the heating efficiency is observed at the FOMT transition temperature of 44^{o}C. The width of the transition is approximately two degrees. Beyond the transition point the nanoparticles are paramagnetic, so that the heating effect is still present (but not zero).

The experimental system for the measurements of the thermal response of the breast tissue model has been fabricated [see Fig.21]. It consists of an agar or gelatin gel enclosed into the hemispherical container and a small volume ($1cm^3$) of the dispersed nano-particles at the center of the container. The RF heating has been recorded in different positions by the thermistors embedded into the agar. The hemispherical container together with the enveloping RF coil is immersed into the water bath maintained at 37^{o}C.

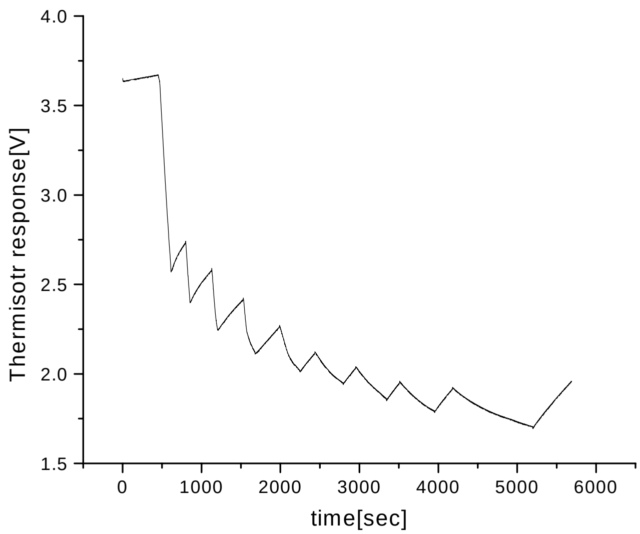

Fig.22. Thermistor response during SAR measuremenrts

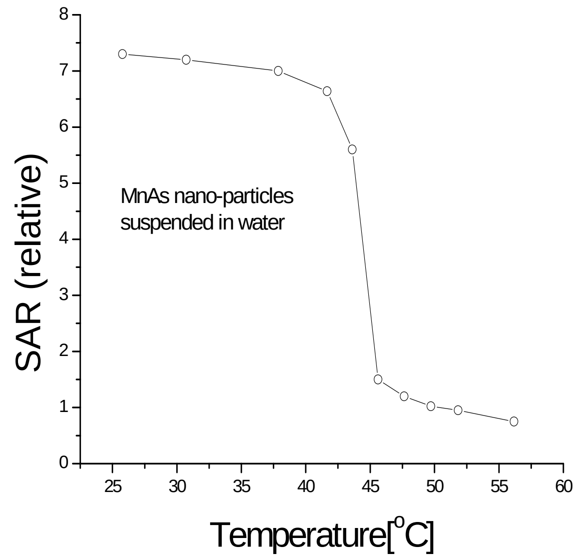

Fig.23. Specific absorption rate of MnAs nanoparticles exhibiting FOMT transition

The Fe_3O_4 vs. MnAs thermo-regulating nanoparticles were compared to validate the thermo-regulating concept. The experimental data were confronted with the theoretical modeling assuming no blood perfusion (agar).

CHARACTERIZATION

This task regularly accompanies the synthesis work. Characterization analysis includes size, composition, magnetic properties, and RF heating properties. The available XRD system is being used for size, and composition analysis. Magnetic measurements were done by the VSM system that gave information about static magnetic properties of the nano-powder. We have realized that state-of-the-art optical characterization can provide valuable information about the size and surface properties of the nano-colloids. This information is critical in assessing the oxidation and capping phenomena. We have acquired an optical dynamic scattering system that could give the hydrodynamic size distribution of the nano-particles. The graph in Fig.9 was obtained in a few minutes, allowing almost in-situ monitoring the synthesis progress. UV-Vis absorption and FTIR spectroscopy has been also investigated as a valuable characterization tool. Atomic force microscopy became available at the late stage of this development that gave useful information about the size and shape of the nanoparticles.

During the course of this research, we have accomplished:

- Performed a series of syntheses of Nickel based, spinels, MnAs, ferrites, and a number of pure metal nano-particles.
- Characterized fabricated nano-particles by measuring their magnetic, optical, and thermo-physical properties.
- Discovered an excellent thermo-regulating class of magnetic materials.
- Demonstrated, experimentally and by numerical modeling, that thermo-regulating properties of MnAs nano-particles are excellent for TR MFH treatment.

As a result of this research effort,

- Two patent applications have been written [1,2].
- Three conference presentations [4-6].
- Three interim reports and one final report.
- One refereed paper [3].
- One proposal applied for based on this research

REPORTABLE OUTCOMES

During the reported period, the final report has been written.
Two research proposals has been applied for.

CONCLUSION

A number of potential systems to produce thermo-regulating magnetic nano-particles has been investigated:

1)The Pt-Ni nano-alloy has been synthesized in the micellar medium. This nano-material, when annealed in a reducing atmosphere, displays predicted magnetic properties. Gold coating of these nanoparticles against oxidizing in air appears problematic.

2)Fe-Ti-O ferrite. Nano-particles of this material have been synthesized in ORNL. They display high magnetization with the Curie temperature above 200^{0}C.

3)Mg-Ti-Fe-O ferrite. This is an attractive biocompatible system. Nano-powders have been fabricated by sintering and ball-milling. They exhibit high magnetization and a good control of the Curie temperature within the 30-60^{o}C range. RF heating of the suspension exhibits expected thermo-regulating property. However, no sharp transition was observed.

4) MnAs. This material exhibits the FOMT transition. Nano-powders of this material have been fabricated by ball-milling. The material exhibits high magnetization even near the transition point. The SAR of this material is excellent for MFH.

Modeling work on breast magnetic fluid hyperthermia has demonstrated that thermo-regulating magnetic fluids offer significant advantages over the ordinary magnetic fluids.

A significant amount of MnAs nano-particles has been fabricated (about 20 gm). It will be sufficient for 1000 MFH applications. This material awaits further *in vivo* MFH and toxicity study.

In order to further evaluate the developed nanoparticle technology, *in vivo* study of toxicity and hyperthermia is required. An idea concept proposal has been submitted to the USAMRMC agency. The proposal has been rejected on the ground that the medical part was not sound. However, the referees liked novelty in nanoparticle technology. The second my attempt was to resubmit the proposal with the new Co-PI –the medical doctor from Duke University, involved in research on hyperthermia. Duke hyperthermia center is the best in US, and they liked my new nanoparticles concept. However, the technical error occurred during the proposal submission. My administrator forgotten to include a doctor's resume into the package. As a result, the proposal was formally rejected by the agency. Last year, I have modified and submitted my proposal to the USAMRMC synergistic award type. The proposal has been rejected again on the ground of lack of synergy and some questions regarding the medical part. Again, the referee panel liked the nanoparticle technology part. At this point, I have resubmitted the proposal to the agency again. In case of rejection, I will find the medical research doctor interested in hyperthermia, and working within one of the NIH cancer institutes. It would be a misfortune if this, so far successful research effort, ends without further *in vivo* studies.

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